THE EFFECT OF LIGHT ON THE SPAWNING OF CIONA INTESTINALIS

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Invertebrate embryologists have long known that a number of ascidians spawn in response to light following darkness. *Molgula manhattensis* (Castle, 1896; Conklin, 1905) and *Ciona intestinalis* (Castle, 1896; Conklin, 1905; Berrill, 1947) normally spawn at dawn but can be induced to spawn at any time by keeping them in the dark until needed; then a short exposure to light causes them to spawn (Costello *et al.*, 1957). *Styela partita* spawns during the late afternoon (Castle, 1896; Conklin, 1905; Rose, 1939). Rose (1939) found that *S. partita* could be induced to spawn at any time by placing them in the dark for 12 hours, then subjecting them to light for 11–12 hours, at the end of which time they spawn.

The physical factors controlling spawning in *Corella parallelogramma* have been extensively investigated by Hŭus (1939, 1941a, 1941b). This ascidian, which normally spawns during the early morning, can be caused to spawn at any hour by exposing dark-adapted animals to the light of a 60-candle bulb 25 cm. from the aquarium for 2 minutes (Hŭus, 1939). Spawning begins within 30 minutes. Hŭus termed this period between illumination and spawning the "dormant period." Limiting temperatures for spawning were found to be 10°–24° C. (Hŭus, 1941a). The duration of the dormant period was determined to be temperature-dependent (1941b), 11 minutes being required at 24° C. and 17 minutes at 10.5° C. Hŭus hypothesized that light causes spawning by eliciting the production of some unknown hormone; the temperature dependency of the dormant period, he stated, tended to support this view.

The present study, on light-induced spawning by *C. intestinalis,* consists of two series of experiments. The first series, using unmeasured white light, determined the minimum reliable dark-adaption time and the time required for spawning after illumination. The second series, using quantified monochromatic light, determined the threshold dose of light energy necessary to cause spawning at different wave-lengths. From these data an action spectrum for spawning is constructed.

Materials and Methods

Experimental animals

Ciona intestinalis between 4 and 7 cm. in overall length were collected in Mission Bay, San Diego, California. Only gravid individuals, identified by their full oviducts, were used in the experiments. Continuous illumination from the time of collection until the dark-adaption period prevented uncontrolled spawning. The animals were used only once, two days after collection.

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Experimental apparatus and procedure

The preliminary experiments used white light from two Sylvania 40-watt daylight fluorescent tubes 147 cm. above the animals. For these experiments, the animals, each in a 400-ml. beaker of sea water, were placed on a 15°–17° C. water table. Dark-adaption for periods of 45 to 60 minutes was accomplished by covering the water table. The animals were either continuously illuminated until spawning occurred, or they were returned to darkness after a one-minute exposure to light.

A Bausch and Lomb high intensity, grating monochrometer (1350 grooves/mm.), equipped with a tungsten (quartz-iodine) 45-watt lamp as light source, was used for monochromatic illumination. The band pass was 10 mμ. The beam from the exit slit (exit lens removed) passed through a leaf shutter, then through the end of the aquarium (10 cm. from monochrometer) to the animal. An approximately circular spot of light, 3 cm. in diameter, was formed on the animal by the exit beam at this distance.

The monochromatic light intensity was measured with phototube C of a Photovolt electronic photometer, model 501-M, placed in the same position relative to the monochrometer as had been the end of the aquarium. The photometer was calibrated at all wave-lengths used against a calibrated Reeder compensated vacuum thermopile (RBL-500) and a Leeds and Northrup (2284b) high sensitivity galvanometer. A National Bureau of Standards 50-watt 115v. secondary Radiometric Standard Lamp was used to calibrate the thermopile-galvanometer system according to the method contained in form NBS-443.

The animals were suspended with nylon string, basal ends upward, in individual 600-ml. beakers of sea water at 16°–17° C. After one hour of dark-adaption the animals were removed from their beakers, placed in the experimental aquarium and illuminated with monochromatic light, one at a time. While being illuminated, the animals were pressed flat against the aquarium end by a flat flask. Since the circle of illumination was only 3 cm. in diameter, only the siphonal end of the animals received the light. After illumination, the animals were returned to their beakers. The beakers were examined for ova 30 minutes after the last animal had been illuminated. Those beakers that contained ova were recorded as positive. Those that did not contain ova were brought into the well-lit laboratory (fluorescent lighting) for 30 minutes, after which time they were again examined for the presence of ova. Beakers that now contained ova but which had not on removal from the darkroom were recorded as negative, that is, the animals were capable of spawning but had not been provoked to spawn by the amount of light energy received.

The duration of exposure at the maximum intensity of a given wave-length necessary to provoke spawning in two out of three animals, when a 10% shorter exposure would not elicit spawning by two out of three animals, was taken as the threshold duration of illumination for spawning at that wave-length. This threshold duration was determined for wave-lengths between 400 mμ and 610 mμ in 15-mμ increments. These values were then converted to threshold doses in quanta/mm^2. An action spectrum was constructed by graphing the reciprocal of the threshold dose against wave-length.

Results and Discussion

White light studies

Dark-adaption periods of 45–55 minutes followed by return to light resulted in spawning by 26 out of 65 animals (40%). One hour of dark-adaption preceding illumination elicited spawning in 47 of the 60 animals tested (78.3%). Following the one-hour dark-adaption period, an average of 27.3 minutes elapsed before spawning occurred. The one-hour dark-adaption period was used for all of the following experiments.

A comparison of these results with *Ciona* and Hŭus's with *Corella* demonstrates clearly that these two ascidians have quite similar spawning responses to light, the main difference being duration of the latent (dormant) period: at 14.5° C., *Corella* spawned 14.5 minutes after exposure to light; *Ciona* spawned 27 minutes after exposure at 15°–16° C.

The observation that *Ciona* spawns at dawn in the laboratory is an old one (Castle, 1896; Conklin, 1905; Berrill, 1947), yet the most recent paper on the spawning of this ascidian (Carlisle, 1951) curiously omits any reference to the light-induced spawning of any ascidian. Carlisle (1951) was investigating the spawning of *Ciona intestinalis* and *Phallusia mammilata* in relation to two other factors: the effect of injecting human chorionic gonadotropin and the effect of ingesting gametes. Carlisle, without discussing the illumination of his laboratory, stated that *Ciona* was never observed to spawn "spontaneously." We are quite confident that in spite of the small number of animals involved in his studies (less than 60), had his laboratory ever been darkened, spawning would have occurred, provided the animals were ripe. Carlisle reported that either injection of chorionic gonadotropin or ingestion of gametes provoked spawning in these two ascidians. This spawning took place 20 hours after treatment, in contrast to the 27-minute latent period established here. Carlisle further states that prior to treatment, no corpora lutea were observed in *Ciona*'s ovary. Millar's (1953) report that the oviduct is always packed with ova prior to spawning has been fully confirmed by our observations. Although the histological structure of the ovary was not examined in this study, the presence of ova in the oviduct implies that corpora lutea should be found in the ovary. A re-evaluation of Carlisle's findings may be made in the light of the observations reported here. Perhaps Carlisle did not provoke spawning by his treatments, but instead induced ovulation. These two phenomena, as demonstrated by the full oviduct prior to spawning, are quite separate in *Ciona*. It should be stated here that Hŭus (1941a) found that *Corella*, prior to spawning, has an empty oviduct, which suggests that in *Corella* spawning and ovulation are either simultaneous or occur closer in time than in *Ciona*.

Monochromatic light studies

The action spectrum for spawning of *C. intestinalis* was obtained by illuminating the animals at different wave-lengths and determining the threshold duration of exposure required to evoke spawning at the maximum intensity of each of these wave-lengths. Since the intensity of the incident beam at each wave-length was known, the quantum requirement (the threshold dose for spawning) was easily calculated.

Since the intensity at each wave-length was different, it is possible that the quantum requirement, determined on the basis of duration of exposure, might have been different if the Reciprocity Law does not hold for some intensities used. However, since the maximum difference in intensity between any two wave-lengths was less than four times (Table I), and since the animals were most sensitive to the wave-lengths showing the lowest intensity, this problem probably does not seriously influence the shape of the action spectrum. Another drawback to this method which became evident as the experiments progressed was that for a reasonable exposure time (5 minutes) the energy output of the monochrometer was too low in the red end of the spectrum to cause spawning. This fact also made it impossible to test for reciprocity, *i.e.*, Intensity × Time equals a Constant Response, at each wave-length used.

TABLE I

Experimental and derived data necessary to establish spawning threshold in quanta

Wave-length mμ	Intensity		Threshold	
	Ergs/sec./mm.²	Quanta × 10^{12}/ sec./mm.²	Duration sec.	Dose Quanta × 10^{14}/mm.²
610	17.64	5.41	660	35.7
595	18.59	5.56	498	27.7
580	14.14	4.12	360	14.9
565	14.62	4.15	88	3.67
550	15.54	4.30	44	1.89
535	14.30	3.86	72	2.77
520	14.00	3.66	66	2.42
505	10.92	2.77	216	6.05
490	13.26	3.27	577	18.9
475	11.52	2.76	378	10.4
460	10.12	2.34	570	13.4
445	8.28	1.86	478	8.89
430	9.90	2.07	56	1.16
415	4.80	1.00	60	0.60
400	4.86	0.978	144	1.41

Table I presents the raw and derived data necessary to obtain the threshold quanta requirements for spawning at all wave-lengths tested.

Of the 884 animals used in this study, 589 (66.6%) spawned in response to light, either after illumination by monochromatic light or after return to the illuminated laboratory.

Action spectrum for spawning

Figure 1 is an action spectrum for photically induced spawning by *Ciona intestinalis*. The reciprocals of the quantum thresholds from Table I are plotted against wave-length to show the relative effectiveness of light of each wave-length in inducing spawning. As can be seen from this figure, there are three peaks of maximum effectiveness. Wave-length 415 mμ is most effective, requiring a dose of light about one-third that of the next most effective wave-length, 550 mμ, to

induce spawning. Wave-lengths 520 mμ and 550 mμ are of nearly equal effectiveness. This action spectrum for spawning by *Ciona intestinalis* suggests that a hemoprotein is the light-absorber because of the great efficiency in the region of the Soret band absorption and the characteristic peaks in the yellow. An examination of the absorption spectra of the hemoproteins led to cytochrome *c* as a possible chromophore.

In Figure 2 the action spectrum for spawning in *Ciona* is replotted as the Relative Effectiveness in Inducing Spawning as a function of wave-length. These data are obtained by setting the reciprocal of the threshold dose in quanta/mm.^{2} at wave-length 415 mμ equal to 100% Relative Effectiveness. The doses at all other wave-lengths are then reduced to a percentage of the dose at 415 mμ. On the

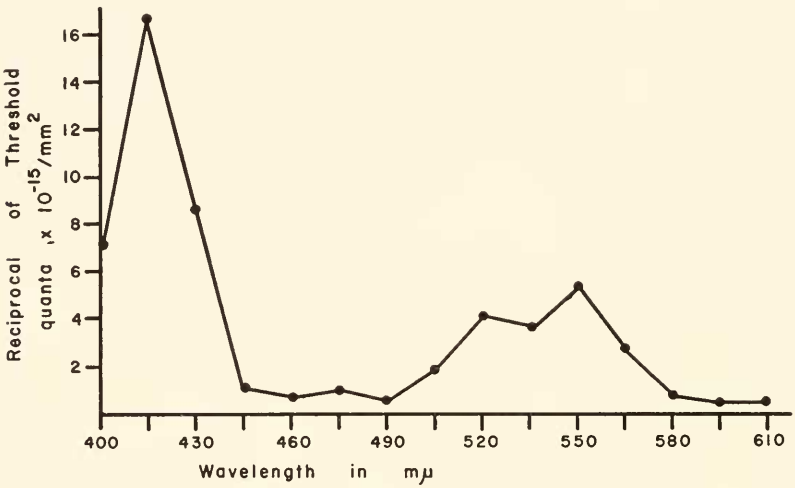

FIGURE 1. Action spectrum for light-induced spawning of *Ciona intestinalis*.

same figure (Fig. 2) are plotted data on the Relative Optical Density of reduced horse heart cytochrome *c*. These data are calculated from those obtained by Margoliash and Frohwirt (1959) by setting the optical density at wave-length 415 mμ equal to 100% Relative Optical Density. At all other wave-lengths, the Relative Optical Density is calculated as a percentage of the optical density at 415 mμ. Comparison of these two curves shows that they are similar in many respects. Oxidation of cytochrome *c* results in the following changes in its absorption spectrum: the major peak at 415 mμ shifts to 410 mμ and is lowered considerably, and the peaks at 520 mμ and 550 mμ are replaced by a single peak at 528 mμ (Margoliash and Frohwirt, 1959). It is evident, therefore, that if cytochrome *c* is the chromophore, it is in the reduced state. The maxima and minima of the action spectrum fit quite well with those of the absorption spectrum. It will be seen, however, that although the heights of the action spectrum maxima are of the same relative order (415 mμ > 550 mμ > 520 mμ) as those of the absorption spectrum, the relative heights at 550 mμ and 520 mμ are different for the two spectra. While the action spectrum for spawning closely matches the reduced cytochrome *c* absorp-

tion spectrum, the resolution attained by our system is not sufficient to do more than suggest that cytochrome *c*, or some other hemoprotein, may be the receptor material.

The role of hemoproteins in photobiological processes has been extensively investigated by Arvanitaki and Chalazonitis (1949, 1960, 1961). These workers, studying the effect of monochromatic light on the visceral ganglion of the gastropod *Aplysia,* have demonstrated that two chromophores are involved in light reception as measured by the electrical activity of isolated neurons. These pigments seem to act in antagonistic ways upon absorption of light. One pigment, a carotene-protein, generally produces a hyperpolarization of the membrane potential and

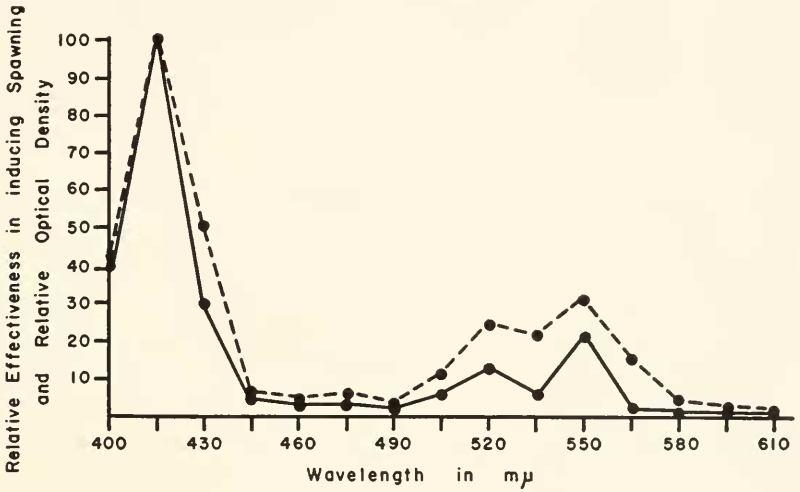

FIGURE 2. A comparison of the absorption spectrum of cytochrome *c* (solid line) with the action spectrum for spawning of *Ciona intestinalis* (dashed line).

inhibition of spiking. The other pigment, a heme-protein, produces a membrane depolarization and the initiation of spiking. The pigments are contained in granules just below the plasma membrane of the nerve cells, imparting a reddish hue to the cells. It is hypothesized (Chalazonitis, 1964) that the heme-protein, upon absorbing light, may pass an electron to the carotene-protein, thereby acting as a photoconductor. This transfer of electrons within the membrane is then visualized as opening channels for ionic flow. Thus a generator current is initiated which, if of sufficient intensity, may initiate action potentials. It is tempting to suggest that light absorbed by heme-proteins in *Ciona* may trigger a similar chain of events leading eventually to spawning. This, of course, implies absorption of light and action at the neuronal level. While it is true that the neural ganglion and numerous nerves of *Ciona* were illuminated in these experiments, other pigmented structures such as the tip of the gonopore and the neural gland, also received light. Studies are now under way to attempt a localization of the light absorbers and to investigate the neurophysiology of this response. Since the visceral ganglion is found deeply buried in the viscera of the intact *Aplysia,* it is extremely unlikely that light

can reach it to cause a behavioral response in such an animal. It is possible that the work reported here on the action spectrum for spawning of *Ciona* is the first demonstration of a hemoprotein involvement in a photo-induced behavioral response by any animal.

Summary

1. The spawning of *Ciona intestinalis* with respect to light was studied, using both white light and monochromatic light.

2. A one-hour dark-adaption period followed by exposure to light resulted in spawning by 66.6% of the 884 animals tested.

3. Spawning occurs an average of 27.3 minutes after the onset of illumination.

4. Illumination need not be continuous until spawning occurs; the animals spawn when returned to the dark after a short illumination period, provided they have received enough energy.

5. The action spectrum for spawning suggests cytochrome *c* as a chromophore.

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